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PATENT- OG VAREMÆRKESTYRELSEN

INTERVENTIONS FOR THE PREVENTION AND TREATMENT OF ALZHEIMER'S AND OTHER AMYLOID DISEASES

FIELD OF THE INVENTION

The present invention relates to a vaccine for the prevention and treatment of Alzheimer's and other amyloid related diseases, which overcomes the drawbacks associated with using naturally occurring peptides, proteins or immunogens, such as full length Beta Amyloid.

The present invention relates to a vaccine for the prevention and treatment of Alzheimer's and other amyloid related diseases.

Amyloidosis refers to a pathological condition characterized by the presence of amyloid fibers. Amyloid is a generic term referring to a group of diverse but specific protein deposits (intracellular and/or extracellular) which are seen in a number of different diseases. Though diverse in their occurrence, all amyloid deposits have common morphologic properties, stain with specific dyes (e.g., Congo red), and have a characteristic red-green birefringent appearance in polarized light after staining. They also share common ultrastructural features and common x-ray diffraction and infrared spectra.

Amyloid-related diseases can either be restricted to one organ or spread to several organs. The first instance is referred to as "localized amyloidosis" while the second is referred to as "systemic amyloidosis".

Some amyloidotic diseases can be idiopathic, but most of these diseases appear as a complication of a previously existing disorder. For example, primary amyloidosis can appear without any other pathology or can follow plasma cell dyscrasia or multiple myeloma. Secondary amyloidosis is usually seen associated with chronic infection (such as tuberculosis) or chronic inflammation (such as rheumatoid arthritis). A familial form of secondary amyloidosis is also seen in Familial Mediterranean Fever (FMF). This familial type of amyloidosis, as one of the other types of familial amyloidosis, is genetically inherited and is found in specific population groups. In these two types of amyloidosis, deposits are found in several organs and are thus considered systemic amyloid diseases. Another type of systemic amyloidosis is found in long-term hemodialysis patients. In each of these cases, a different amyloidogenic protein is

involved in amyloid deposition.

"Localized amyloidoses" are those that tend to involve a single organ system. Different amyloids are also characterized by the type of protein present in the deposit. For example, neurodegenerative diseases such as scrapie, bovine spongiform encephalitis, Creutzfeldt-Jakob disease and the like are characterized by the appearance and accumulation of a protease-resistant form of a prion protein (referred to as AScr or PrP-27) in the central nervous system. Similarly, Alzheimer's disease, another neurodegenerative disorder, is characterized by neuritic plaques and neurofibrillary tangles. In this case, the plaque and blood vessel amyloid is formed by the deposition of fibrillar A.beta. amyloid protein. Other diseases such as adult-onset diabetes (Type II diabetes) are characterized by the localized accumulation of amyloid in the pancreas.

Once these amyloids have formed, there is no known, widely accepted therapy or treatment which significantly dissolves the deposits in situ.

Each amyloidogenic protein has the ability to organize into .beta.-sheets and to form insoluble fibrils which get deposited extracellularly or intracellularly. Each amyloidogenic protein, although different in amino acid sequence, has the same property of forming fibrils and binding to other elements such as proteoglycan, amyloid P and complement component. Moreover, each amyloidogenic protein has amino acid sequences which, although different, will show similarities such as regions with the ability to bind to the glycosaminoglycan (GAG) portion of proteoglycan (referred to as the GAG binding site) as well as other regions which will promote .beta.-sheet formation.

In specific cases, amyloidotic fibrils, once deposited, can become toxic to the surrounding cells. As per example, the A.beta. fibrils organized as senile plaques have been shown to be associated with dead neuronal cells and microgliosis in patients with Alzheimer's disease. When tested in vitro, A.beta. peptide was shown to be capable of triggering an activation process of microglia (brain macrophages), which would explain the presence of microgliosis and brain inflammation found in the brain of patients with Alzheimer's disease.

In another type of amyloidosis seen in patients with Type II diabetes, the amyloidogenic

protein IAPP has been shown to induce .beta.-islet cell toxicity in vitro. Hence, appearance of IAPP fibrils in the pancreas of Type II diabetic patients could contribute to the loss of the .beta. islet cells (Langerhans) and organ dysfunction.

People suffering from Alzheimer's disease develop a progressive dementia in adulthood, accompanied by three main structural changes in the brain: diffuse loss of neurons in multiple parts of the brain; accumulation of intracellular protein deposits termed neurofibrillary tangles; and accumulation of extracellular protein deposits termed amyloid or senile plaques, surrounded by misshapen nerve terminals (dystrophic neurites). A main constituent of these amyloid plaques is the amyloid-.beta. peptide (A.beta.), a 40-42 amino-acid protein that is produced through cleavage of the .beta.-amyloid precursor protein (APP). Although symptomatic treatments exist for Alzheimer's disease, this disease cannot be prevented nor cured at this time.

The use of a vaccine to treat Alzheimer's disease is possible in principle (Schenk, D. et al., (1999) Nature 400, 173-177). Schenk et al. show that, in a transgenic mouse model of brain amyloidosis (as seen in Alzheimer's disease), immunization with A.beta. peptide inhibits the formation of amyloid plaques and the associated dystrophic neurites. In that study, a vaccine using the native human peptide as immunogen prevented the formation of .beta.-amyloid plaque, astrogliosis and neuritic dystrophy in vaccinated transgenic mice.

However, it is apparent that there are a number of drawbacks to using an endogenous protein as a vaccine (or a protein naturally present in the animal being vaccinated). Some of these drawbacks include:

Possible development of autoimmune disease due to the generation of antibodies against "self" protein.

Difficulty in eliciting an immune response due to the failure of the host immune system to recognize "self" antigens.

Possible development of an acute inflammatory response.

SUMMARY OF THE INVENTION

The present invention relates to a vaccine for the prevention and treatment of Alzheimer's and other amyloid related diseases, which overcomes the drawbacks associated with using naturally occurring peptides, proteins or immunogens, such as full length Beta Amyloid.

The term "amyloid peptide" as used herein, encompasses both monomeric and oligomeric beta-amyloid or any fibril protein as discussed herein, as well as any other structural variants that may occur naturally, are synthetically constructed or correspond to a known protein. Specifically, an amyloid peptide consists of at least 3 amino acids from a fibril peptide, such as beta amyloid or any structural variant thereof.

The vaccines of the present invention may be used in the prevention and/or treatment of amyloid related diseases, and in the manufacture of medicaments for preventing and/or treating amyloid-related diseases. The term "amyloid related diseases" includes diseases associated with the accumulation of amyloid which can either be restricted to one organ, "localized amyloidosis", or spread to several organs, "systemic amyloidosis". Secondary amyloidosis may be associated with chronic infection (such as tuberculosis) or chronic inflammation (such as rheumatoid arthritis), including a familial form of secondary amyloidosis which is also seen in Familial Mediterranean Fever (FMF) and another type of systemic amyloidosis found in long-term hemodialysis patients. Localized forms of amyloidosis include, without limitation, diabetes type II and any related disorders thereof, neurodegenerative diseases such as scrapie, bovine spongiform encephalitis, Creutzfeldt-Jakob disease, Alzheimer's disease, Cerebral Amyloid Angiopathy, and prion protein related disorders.

An amyloid peptide or protein can be derived from precursor protein known to be associated with certain forms of amyloid diseases. Such precursor proteins include, but are not limited to, Serum Amyloid A protein (ApoSSA), immunoglobulin light chain, immunoglobulin heavy chain, ApoA1, transthyretin, lysozyme, fibrinogen .alpha. chain, gelsolin, cystatin C, Amyloid beta protein precursor (beta.-APP), Beta.sub.2 microglobulin, prion precursor protein (PrP), atrial natriuretic factor, keratin, islet amyloid polypeptide, a peptide hormone, and synuclein. Such precursors also include modified peptides, mutant proteins, protein fragments and proteolytic peptides of such precursors.

In a preferred embodiment, the invention is effective to induce an immune response directed against the carboxyl-terminal of an amyloid peptide. In another embodiment, the invention is effective to induce an immune response directed against an epitope formed by a fibril or amyloid protein or peptide. The term "epitope" refers to a site on an antigen to which B and/or T cells respond. B-cell epitopes can be formed both from contiguous amino acids or noncontiguous amino acids juxtaposed by tertiary folding of a protein. Epitopes formed from contiguous amino acids are typically retained on exposure to denaturing solvents whereas epitopes formed by tertiary folding are typically lost on treatment with denaturing solvents. An epitope typically includes at least 3, and more usually, at least 5 or 8-10 amino acids in a unique spatial conformation.

Accordingly, in one embodiment of the present invention, a vaccine is provided which is produced using a Ligand Presenting Assembly (LPA e.g. as described in WO00/18791) backbone to link and present the carboxyl-terminal(s) of one or several amyloid peptide(s) or fragments or derivatives thereof. Such peptides may be composed of naturally occurring amino acids or synthesized from unnatural amino acids. The peptides need not be aggregated to be operative or immunogenic as opposed to the prior art vaccines.

In another embodiment, there is provided a method for preventing and/or treating an amyloid-related disease in a subject, by administering to the subject an antigenic amount of a peptide which elicits production of antibodies specifically directed against the carboxyl-terminal of an amyloid peptide, therefore preventing fibrillogenesis, associated cellular toxicity and neurodegeneration. These vaccines may be used in the prevention and/or treatment of amyloid related diseases, and in the manufacture of medicaments for preventing and/or treating amyloid-related diseases.

In a further embodiment of the invention, a vaccine for preventing and/or treating an amyloid-related disease in a subject comprises at least one antibody which interacts with amyloid proteins to prevent fibrillogenesis, wherein the antibodies are raised against an antigenic amount of carboxyl-terminally exposed peptide, e.g. A.beta.(1-42) or immunogenic fragments thereof, protein conjugates thereof, immunogenic derivative peptides thereof, immunogenic peptides thereof, and immunogenic peptidomimetics thereof, or a peptide which has a substantial identity to any of the above peptides.

In a further embodiment of the invention, a vaccine for preventing and/or treating an amyloid-related disease in a subject comprises at least one antibody which interacts with amyloid proteins to prevent fibrillogenesis, wherein the antibodies are raised against one or more carboxyl-terminally exposed peptide(s) in conjunction to a LPA backbone. The exposed peptide can be e.g. A.beta.(1-42) or immunogenic fragments thereof, protein conjugates thereof, immunogenic derivative peptides thereof, immunogenic peptides thereof, and immunogenic peptidomimetics thereof, or a peptide which has a substantial identity to any of the above peptides. When more than one peptide is linked to the same LPA backbone the peptides may be identical or different

The term "substantial identity" means that two peptide sequences share at least 50 percent sequence identity, preferably at least 60 percent sequence identity, more preferably at least 70 percent sequence identity, more preferably at least 80 percent sequence identity, more preferably at least 90 percent sequence identity, more preferably at least 95 percent sequence identity or more (e.g., 99 percent sequence identity). Preferably, residue positions which are not identical differ by conservative amino acid substitutions. Conservative amino acid substitutions refer to the interchangeability of residues having similar side chains. For example, a group of amino acids having aliphatic side chains is glycine, alanine, valine, leucine, and isoleucine; a group of amino acids having aliphatic-hydroxyl side chains is serine and threonine; a group of amino acids having amide-containing side chains is asparagine and glutamine; a group of amino acids having aromatic side chains is phenylalanine, tyrosine, and tryptophan; a group of amino acids having basic side chains is lysine, arginine, and histidine; and a group of amino acids having sulfur-containing side chains is cysteine and methionine. Preferred conservative amino acids substitution groups are: valine-leucine-isoleucine, phenylalanine-tyrosine, lysine-arginine, alanine-valine, and asparagine-glutamine.

In another related aspect, the invention includes a method of preventing or treating a disorder characterized by amyloid deposition in a mammalian subject. In accordance with this aspect of the invention, the subject is given a dosage of a peptide effective to produce an immune response against an amyloid peptide characteristic of the amyloid disorder from which the subject suffers. Essentially, the methods include administering pharmaceutical compositions containing immunogenic amyloid peptides specific to the disorder, either alone or conjugated to a LPA backbone such as those described above. Such methods are further characterized by their effectiveness in inducing a

specific immunogenic responses in the subject. According to a preferred embodiment, the method is effective to produce an immunological response that is characterized by a serum titer of at least 1:1000 with respect to the amyloid peptide against which the immunogenic peptide is directed. In yet a further preferred embodiment, the serum titer is at least 1:5000 with respect to the fibril component. According to a related embodiment, the immune response is characterized by a serum amount of immunoreactivity corresponding to greater than about four times higher than a serum level of immunoreactivity measured in a pre-treatment control serum sample. This latter characterization is particularly appropriate when serum immunoreactivity is measured by ELISA techniques, but can apply to any relative or absolute measurement of serum immunoreactivity. According to a preferred embodiment, the immunoreactivity is measured at a serum dilution of about 1:100.

According to a still related aspect, the invention also includes so-called "passive immunization" methods and pharmaceutical compositions for preventing or treating amyloid diseases. According to this aspect of the invention, patients are given an effective dosage of an antibody that specifically binds to a selected amyloid peptide. In general, such antibodies are selected for their abilities to specifically bind the various proteins, peptides, and components described with respect to the pharmaceutical compositions and methods described above. In a related embodiment, such methods and compositions may include combinations of antibodies that bind at least two amyloid fibril components. The antibody can also be a monoclonal antibody.

As used herein, the term "compound" refers to a peptide of the present invention or a pharmaceutically acceptable composition containing a peptide according to the present invention. In a preferred embodiment of the present invention, the compound is a compound of Formula I:

$R'-L-(P)_n$ (I)

wherein

P is an amyloid peptide, e.g., beta. sheet region, GAG-binding site region, A.beta.(e.g. 1-42), and macrophage adherence region (10-16) immunogenic fragments thereof, immunogenic derivatives thereof, protein conjugates thereof, immunogenic peptides

thereof, and immunogenic peptidomimetics thereof;

n is a whole number higher than or similar to 1

L is a backbone e.g. as described in WO00/18791 e.g. $N(CH_2CO)_2$

R' is an N-terminal substituent, e.g.: hydrogen; lower alkyl groups, e.g., acyclic or cyclic having 1 to 8 carbon atoms, without or with functional groups, e.g., carboxylate, sulfonate and phosphonate; aromatic groups; heterocyclic groups; acyl groups, e.g., alkylcarbonyl, arylcarbonyl, sulfonyl and phosphonyl groups; and peptidic group.

In one embodiment, R' is a human T cell epitope, e.g. the tetanus toxoid H-FNNFTVSFWLRVPKVSASHLE or a rodent T cell epitope H-QYIKANSKFIGITEL (as described by Valmori et al.).

In one embodiment, P is an amyloid peptide or fragment thereof or a peptide with substantial similarity to an amyloid peptide or a fragment thereof. In a preferred embodiment of the present invention, the subject is a human being. In yet another embodiment of the present invention, the amyloid related disease may be Alzheimer's disease. In still another embodiment of the present invention, the amyloid related disease may be Cerebral Amyloid Angiopathy (CAA).

In another embodiment of the present invention, there is provided a method for preventing and/or treating of an amyloid related disease in a subject, comprising administering to the subject an antigenic amount of a compound of Formula II:

$$R'\text{-}L\text{-}(P`)_n \quad (II)$$
$$(P^*)_n$$

wherein

P` is a an amyloid peptide as P described above under (I) and P* is an amyloid peptide as described above under (I), but where P`is not identical to P*

L and R' and n are as described above under (I)

In accordance with a preferred embodiment of the present invention, some methods entail administering a dosage to a subject of a peptide according to (I) or (II) that is effective to produce an immune response against an amyloid peptide characteristic of the amyloid disorder from which the subject suffers. In one preferred embodiment, the administration of the peptide will result in an antibody response that specifically binds to the 1-42 version of A.beta, preferably to residues 25-42, more preferably to residues 35-42.

In another embodiment, the antibody response binds specifically to the carboxyl terminal of the 1-39 version of A.beta, or to the 1-40 version of A.beta or to the 1-43 version of A.beta. In yet another embodiment, the antibody response specifically binds to an epitope within residues 15-20 of A.beta.. In some methods, the antibody response specifically binds to an epitope within residues 13-21 of A.beta.. In some methods, the antibody response specifically binds to an epitope within residues 10-21 of A.beta.. In some methods, the antibody response specifically binds to an epitope within residues 10-16 of A.beta.. In some methods, the antibody response specifically binds to an epitope within residues 25-35 of A.beta.

The methods can be used on both asymptomatic patients and those currently showing symptoms of disease.

The present invention also relates to any use of antibodies generated by the methods described

Other features and advantages of the invention will be apparent from the detailed description and claims.

DETAILED DESCRIPTION OF THE INVENTION

Amyloid diseases or amyloidoses include a number of disease states having a wide variety of outward symptoms. These disorders have in common the presence of abnormal extracellular deposits of protein fibrils, known as "amyloid deposits" or "amyloid plaques" that are usually about 10-100 nm in diameter and are localized to specific organs or tissue regions. Such plaques are composed primarily of a naturally occurring soluble protein or peptide. These insoluble deposits are composed of generally lateral aggregates of fibrils that are approximately 10-15 nm in diameter. Amyloid fibrils produce a characteristic apple green birefringence in polarized light,

when stained with Congo Red dye.

The peptides or proteins forming the plaque deposits are often produced from a larger precursor protein. More specifically, the pathogenesis of amyloid fibril deposits generally involves proteolytic cleavage of an "abnormal" precursor protein into fragments. These fragments generally aggregate into anti-parallel .beta.-pleated sheets; however, certain undegraded forms of precursor protein have been reported to aggregate and form fibrils in familial amyloid polyneuropathy (variant transthyretin fibrils) and dialysis-related amyloidosis.

Specifically, the A.beta.(1-42) is known to display a strong tendency to rapidly form amyloid fibrils, whereas shorter forms of amyloid beta peptides have a significantly weaker tendency to form fibrils. It is therefore likely that A.beta (1-42) is catalyzing the formation of amyloid fibrils in Alzheimer's disease and by specifically eliminating A.beta (1-42) immunologically it may be possible to prevent or alleviate the pathology of Alzheimer's disease. Other amyloid proteins which may be used in the present invention include, without limitation, the beta sheet region of IAPP (24-29, all-D), .beta.2-microglobulin, amyloid A protein, and prion-related proteins.

The disorders are classified on the basis of the major fibril components forming the plaque deposits, as discussed below.

Amyloid Diseases

The present invention is based on the discovery that amyloid diseases can be treated by administering peptides that serve to stimulate an immune response against a component or components of the various disease-specific amyloid deposits. The sections below serve to exemplify major forms of amyloidosis and are not intended to limit the invention.

AA (Reactive) Amyloidosis

Generally, AA amyloidosis is a manifestation of a number of diseases that provoke a sustained acute phase response. Such diseases include chronic inflammatory disorders, chronic local or systemic microbial infections, and malignant neoplasms.

AA fibrils are generally composed of 8000 dalton fragments (AA peptide or protein) formed by proteolytic cleavage of serum amyloid A protein (apoSSA), a circulating apolipoprotein which is present in HDL complexes and which is synthesized in hepatocytes in response to such cytokines as IL-1, IL-6 and TNF. Deposition can be widespread in the body, with a preference for parenchymal organs. The spleen is usually a deposition site, and the kidneys may also be affected. Deposition is also common in the heart and gastrointestinal tract.

AA amyloid diseases include, but are not limited to inflammatory diseases, such as rheumatoid arthritis, juvenile chronic arthritis, ankylosing spondylitis, psoriasis, psoriatic arthropathy, Reiter's syndrome, Adult Still's disease, Behcet's syndrome, and Crohn's disease. AA deposits are also produced as a result of chronic microbial infections, such as leprosy, tuberculosis, bronchiectasis, decubitus ulcers, chronic pyelonephritis, osteomyelitis, and Whipple's disease. Certain malignant neoplasms can also result in AA fibril ainyloid deposits. These include such conditions as Hodgkin's lymphoma, renal carcinoma, carcinomas of gut, lung and urogenital tract, basal cell carcinoma, and hairy cell leukemia.

AL Amyloidoses

AL amyloid deposition is generally associated with almost any dyscrasia of the B lymphocyte lineage, ranging from malignancy of plasma cells (multiple myeloma) to benign monoclonal gammopathy. At times, the presence of amyloid deposits may be a primary indicator of the underlying dyscrasia.

Fibrils of AL amyloid deposits are composed of monoclonal immunoglobulin light chains or fragments thereof. More specifically, the fragments are derived from the N-terminal region of the light chain (kappa or lambda) and contain all or part of the variable (V.sub.L) domain thereof. Deposits generally occur in the mesenchymal tissues, causing peripheral and autonomic neuropathy, carpal tunnel syndrome, macroglossia, restrictive cardiomyopathy, arthropathy of large joints, immune dyscrasias, myelomas, as well as occult dyscrasias. However, it should be noted that almost any tissue, particularly visceral organs such as the heart, may be involved.

Hereditary Systemic Amyloidoses

There are many forms of hereditary systemic amyloidoses. Although they are relatively rare conditions, adult onset of symptoms and their inheritance patterns (usually autosomal dominant) lead to persistence of such disorders in the general population. Generally, the syndromes are attributable to point mutations in the precursor protein leading to production of variant asnyloidogenic peptides or proteins. Without limiting the scope of the invention, some prominent example of this group is described in the following.

More than 40 separate point mutations in the transthyretin gene have been described, all of which give rise to clinically similar forms of familial amyloid polyneuropathy. Transthyretin (TTR) is a 14 kilodalton protein that is also sometimes referred to as prealbumin. It is produced by the liver and choroid plexus, and it functions in transporting thyroid hormones and vitamin A. At least 50 variant forms of the protein, each characterized by a single amino acid change, are responsible for various forms of familial amyloid polyneuropathy. For example, substitution of proline for leucine at position 55 results in a particularly progressive form of neuropathy; substitution of methionine for leucine at position 111 resulted in a severe cardiopathy in Danish patients. Amyloid deposits isolated from heart tissue of patients with systemic amyloidosis have revealed that the deposits are composed of a heterogeneous mixture of TTR and fragments thereof, collectively referred to as ATTR, the full length sequences of which have been characterized. ATTR fibril components can be extracted from such plaques and their structure and sequence determined according to the methods known in the art (e.g., Gustavsson, A., et al., Laboratory Invest. 73: 703-708, 1995; Kametani, F., et al., Biochem. Biophys. Res. Commun. 125: 622-628, 1984; Pras, M., et al., PNAS 80: 539-42, 1983).

Persons having point mutations in the molecule apolipoprotein AI (e.g., Gly.fwdarw.Arg26; Trp 4.fwdarw.Arg50; Leu.fwdarw.4 Arg60) exhibit a form of amyloidosis ("stertag type") characterized by deposits of the protein apolipoprotein AI or fragments thereof (AApoAI). These patients have low levels of high density lipoprotein (HDL) and present with a peripheral neuropathy or renal failure.

A mutation in the alpha chain of the enzyme lysozyme (e.g., Ile.fwdarw.Thr56 or Asp.fwdarw.His57) is the basis of another form of stertag-type non-neuropathic hereditary amyloid reported in English families. Here, fibrils of the mutant lysozyine protein (Alys) are deposited, and patients generally exhibit impaired renal function. This

protein, unlike most of the fibril-forming proteins described herein, is usually present in whole (unfragmented) form (Benson, M. D., et al. CIBA Fdn. Symp. 199: 104-131, 1996).

beta.-amyloid peptide (A.beta.) is a 39-43 amino acid peptide derived by proteolysis from a large protein known as Beta Amyloid Precursor protein (.beta.APP). Mutations in .beta.APP result in familial forms of Alzheimer's disease, Down's syndrome and/or senile dementia, characterized by cerebral deposition of plaques composed of .beta.P fibrils and other components, which are described in further detail below. Known mutations in APP associated with Alzheimer's disease occur proximate to the cleavage sites of .beta. or gamma-secretase, or within A.beta.. For example, position 717 is proximate to the site of gamma-secretase cleavage of APP in its processing to A.beta., and positions 670/671 are proximate to the site of .beta.-secretase cleavage. Mutations at any of these residues may result in Alzheimer's disease, presumably by causing an increase the amount of the highly amyloidogenic 42/43 amino acid form of A.beta. generated from APP. The structure and sequence of A.beta. peptides of various lengths are well known in the art. Such peptides can be made according to methods known in the art (e.g., Glenner and Wong, Biochem Biophys. Res. Comm. 129: 885-890, 1984; Glenner and Wong, Biochem Biophys. Res. Comm. 122: 113 1-1135, 1984). In addition, various forms of the peptides are commercially available.

Synuclein is a synapse-associated protein that resembles an alipoprotein and is abundant in neuronal cytosol and presynaptic terminals. A peptide fragment derived from alpha-synuclein, termed NAC, is also a component of amyloid plaques of Alzheimer's disease. (Clayton, et al., 1998). This component also serves as a target for immunologically-based treatments of the present invention, as detailed below.

Gelsolin is a calcium binding protein that binds to fragments and actin filaments. Mutations at position 187 (e.g., Asp.fwdarw.Asn; Asp.fwdarw.Tyr) of the protein result in a form of hereditary systemic amyloidosis, usually found in patients from Finland, as well as persons of Dutch or Japanese origin. In afflicted individuals, fibrils formed from gelsolin fragments (Agel), usually consist of amino acids 173-243 (68 kDa carboxyterminal fragment) and are deposited in blood vessels and basement membranes, resulting in corneal dystrophy and cranial neuropathy which progresses to peripheral neuropathy, dystrophic skin changes and deposition in other organs. (Kangas, H., et al. Human Mol. Genet. 5(9): 1237-1243, 1996).

Other mutated proteins, such as mutant alpha chain of fibrinogen (AfibA) and mutant cystatin C (Acys) also form fibrils and produce characteristic hereditary disorders. AfibA fibrils form deposits characteristic of a nonneuropathic hereditary amyloid with renal disease; Acys deposits are characteristic of a hereditary cerebral amyloid angiopathy reported in Iceland. (Isselbacher, Ct at., Harrison's Principles of Internal Medicine, McGraw-Hill, San Francisco, 1995; Benson, et al., supra.). In at least some cases, patients with cerebral amyloid angiopathy (CAA) have been shown to have amyloid fibrils containing a non-mutant form of cystatin C in conjunction with beta protein. (Nagai, A., et al. Molec. Chem. Neuropathol. 33: 63-78, 1998).

Certain forms of prion disease are now considered to be heritable, accounting for up to 15% of cases, which were previously thought to be predominantly infectious in nature. (Baldwin, et al., in Research Advances in Alzheimer's Disease and Related Disorders, John Wiley and Sons, New York, 1995). In such prion disorders, patients develop plaques composed of abnormal isoforms of the normal prion protein (PrP.sup.Sc). A predominant mutant isoform, PrP.sup.Sc, also referred to as AScr, differs from the normal cellular protein in its resistance to protease degradation, insolubility after detergent extraction, deposition in secondary lysosomes, post-translational synthesis, and high .beta.-pleated sheet content. Genetic linkage has been established for at least five mutations resulting in Creutzfeldt-Jacob disease (CJD), Gerstmann-Strussler-Scheinker syndrome (GSS), and fatal familial insomnia (FFI). (Baldwin) Methods for extracting fibril peptides from scrapie fibrils, determining sequences and making such peptides are known in the art. (e.g., Beekes, M., et al. J. Gen. Virol. 76: 2567-76, 1995).

Senile Systemic Amyloidosis

Amyloid deposition, either systemic or focal, increases with age. For example, fibrils of wild type transthyretin (TTR) are commonly found in the heart tissue of elderly individuals. These may be asymptomatic, clinically silent, or may result in heart failure. Asymptomatic fibrillar focal deposits may also occur in the brain (A.beta.), corpora amylacea of the prostate (A.beta..sub.2 microglobulin), joints and seminal vesicles.

Cerebral Amyloidosis

Local deposition of amyloid is common in the brain, particularly in elderly individuals. The most frequent type of amyloid in the brain is composed primarily of A.beta. peptide fibrils, resulting in dementia or sporadic (non-hereditary) Alzheimer's disease. In fact, the incidence of sporadic Alzheimer's disease greatly exceeds forms shown to be hereditary. Fibril peptides forming these plaques are very similar to those described above, with reference to hereditary forms of Alzheimer's disease (AD).

Dialysis-related Amyloidosis

Plaques composed of .beta..sub.2 microglobulin (A.beta..sub.2M) fibrils commonly develop in patients receiving long term hemodialysis or peritoneal dialysis. .beta..sub.2 microglobulin is a 11.8 kilodalton polypeptide and is the light chain of Class I MHC antigens, which are present on all nucleated cells. Under normal circumstances, it is continuously shed from cell membranes and is normally filtered by the kidney. Failure of clearance, such as in the case of impaired renal function, leads to deposition in the kidney and other sites (primarily in collagen-rich tissues of the joints). Unlike other fibril proteins, A.beta..sub.2M molecules are generally present in unfragmented form in the fibrils. (Benson, supra).

Hormone-derived Amyloidoses

Endocrine organs may harbor amyloid deposits, particularly in aged individuals. Hormone-secreting tumors may also contain hormone-derived amyloid plaques, the fibrils of which are made up of polypeptide hormones such as calcitonin (medullary carcinoma of the thyroid), islet amyloid polypeptide (amylin; occurring in most patients with Type II diabetes), and atrial natriuretic peptide (isolated atrial amyloidosis). Sequences and structures of these proteins are well known in the art.

Miscellaneous Amyloidoses

There are a variety of other forms of amyloid disease that are normally manifest as localized deposits of amyloid. In general, these diseases are probably the result of the localized production and/or lack of catabolism of specific fibril precursors or a predisposition of a particular tissue (such as the joint) for fibril deposition. Examples of such idiopathic deposition include nodular AL amyloid, cutaneous amyloid, endocrine

amyloid, and tumor-related amyloid.

Pharmaceutical Compositions

The pharmaceutical compositions of the present invention are intended for vaccines comprising antigenic compounds containing full length, fragments, modified versions or derivatives of amyloid fibril peptides. Preferably, the vaccines are prepared from A.beta.(10-21), A.beta.(13-21), A.beta.(25-35), A.beta.(16-21), A.beta.(10-16), A.beta.(1-40), A.beta.(1-42) or the C-terminal regions of A.beta.(1-42), is believed to elicit an immune response in the host or in producing antibodies that recognize the naturally occurring target. Modified A.beta peptides may also be applied, but similarity to native A.beta (1-42) should be greater than or equal to 50%; 55%; 60%; 65%; 70%;75%; 80%; 85%; 90%; 95% or 100%

The vaccine according to the present invention is able to prevent the development of brain amyloidosis through two possible scenerios: 1) the effect of anti-A.beta. antibodies at the site of amyloid deposition, and 2) the systemic effect of the high circulatory anti-A.beta. level on the plasmatic A.beta. concentrations.

Specifically, elevated plasma anti-A.beta. antibody levels may act systemically by decreasing normal A.beta. plasma levels, thereby creating a systemic imbalance in the normal A.beta. levels. Such an imbalance could lead to the activation of the mechanism responsible for the clearing in A.beta. levels from the brain, in order to re-establish the normal balance between brain and plasma A.beta. levels.

Accordingly, this possibility could be exploited by determining the effect of active or passive immunization on plasma and brain levels of A.beta.40 and A.beta 42 at different timepoints following such immunization. A.beta.-immunization can also exert a systemic protective effect versus the development of brain amyloidosis. The ratio of A.beta. levels in plasma and brain should remain constant in immunized transgenic animals, while it should decrease in the control animals. Additionally, B-cell or bone marrow cell transfer from immunized to naive transgenic animals should have the same effect as passive immunization using anti-A.beta. antibodies.

Furthermore, the vaccine of the present invention does not need to be aggregated to

induce an immune response. Pharmaceutical compositions of the present invention may include, in addition to the immunogenic peptide(s), an effective T cell epitope either covalently linked to the immunogenic peptide or non-covalently. Pharmaceutical compositions of the present invention may further include an effective amount of an adjuvant and/or an excipient. Pharmaceutically effective and useful adjuvants and excipients are well known in the art, and are described in more detail below.

According to the present invention, compositions capable of eliciting or providing an immune response directed to certain components of amyloid plaques are effective to treat or prevent development of amyloid diseases. In particular, according to the invention provided herein, it is possible to prevent progression of, ameliorate the symptoms of, and/or reduce amyloid plaque burden in afflicted individuals, when an immunostimulatory dose of an anti-amyloid peptide, or corresponding anti-amyloid immune repeptide, is administered to the patient. This section describes exemplary anti-amyloid peptides that may produce active, as well as passive, immune responses to amyloid plaques.

Antibodies, Analogs and Fragments of Amyloid Proteins

Generally, antigen peptides of the invention are composed of a specific plaque component, preferably a fibril forming and highly amyloidogenic component, which is usually a characteristic protein, peptide, or fragment thereof. For instance, .beta.-amyloid peptide can be used in any of its naturally occurring forms. The human forms of A.beta. are referred to as A.beta.39, A.beta.40, A.beta.41, A.beta.42 and A.beta.43. The sequences of these peptides and their relationship to the APP precursor are illustrated by FIG. 1 of Hardy et al., TINS 20, 155-158 (1997). For example, A.beta.42 has the sequence:

2 H2N-Asp-Ala-Glu-Phe-Arg-His-Asp-Ser-Gly-Tyr-Glu-Val-His-His-Gln-Lys-Leu-Val-Phe-Phe-Ala-Glu-Asp-Val-Gly-Ser-Asn-Lys-Gly-Ala-Ile-Ile-Gly-Leu-Met-Val-Gly-Gly-Val-Val-Ile-Ala-OH

A.beta.41, A.beta.40 and A.beta.39 differ from A.beta.42 by the omission of Ala, Ala-Ile and Ala-Ile-Val respectively from the C-terminal end. A.beta.43 differs from A.beta.42 by the presence of a threonine residue at the C-terminus.

Some immunogenic fragments of A.beta. have a sequence of at least 2, 3, 5, 6, 10 or 20 contiguous amino acids from a natural peptide. Some immunogenic fragments have no more than 10, 9, 8, 7, 5 or 3 contiguous residues from A.beta.. Preferred immunogenic fragments include derivatives of the carboxyl terminus of residues 1-42 of A.beta.. In some methods, the antibody specifically binds to an epitope within residues 15-20 of A.beta.. In some methods, the antibody specifically binds to an epitope within residues 13-21 of A.beta.. In some methods, the antibody specifically binds to an epitope within residues 10-21 of A.beta.. In some methods, the antibody specifically binds to an epitope within residues 10-16 of A.beta.. In some methods, the antibody specifically binds to an epitope within residues 25-35 of A.beta.. The designation A.beta.15-20 for example, indicates a fragment including residues 15-20 of A.beta. and lacking other residues of A.beta.. Other less preferred fragments include A.beta.1-5, 1-6, 1-7, 1-10, 3-7, 1-3, 1-4 and peptides lacking at least three, and sometimes at least 4, 5 or 10 of the C-terminal amino acids present in a naturally occurring A.beta.1-42. Other components of amyloid plaques, for example, synuclein, and epitopic fragments thereof can also be used to induce an immunogenic response.

Unless otherwise indicated, reference to A.beta. includes the natural human amino acid sequences indicated above as well as analogs including allelic, species and induced variants. Analogs typically differ from naturally occurring peptides at one, two or a few positions, often by virtue of conservative substitutions. Analogs typically exhibit at least 50% sequence identity with natural peptides, and preferably 60%, 70%, 80% and most preferably 90% sequence identity. Some analogs also include unnatural amino acids or modifications of N or C terminal amino acids at a one, two or a few positions. For example, the natural aspartic acid residue at position 1 and/or 7 of A.beta. can be replaced with iso-aspartic acid. Examples of unnatural amino acids are D-amino acids, .alpha.,.alpha.-disubstituted amino acids, N-alkyl amino acids, lactic acid, 4-hydroxyproline, y-carboxyglutamate, .epsilon.-N,N,N-trimethyllysi- ne, .epsilon.-N-acetyllysine, O-phosphoserine, N-acetylserine, N-formylmethionine, 3-methylhistidine, 5-hydroxylysine, .omega.-N-methylarginine, and isoaspartic acid. Fragments and analogs can be screened for prophylactic or therapeutic efficacy in transgenic animal models in comparison with untreated or placebo controls as described below.

A.beta., its fragments, and analogs can be synthesized by solid phase peptide synthesis or recombinant expression, or can be obtained from natural sources. Automatic peptide synthesizers are commercially available from numerous suppliers,

such as Applied Biosystems, Foster City, Calif. Recombinant expression can be in bacteria, such as E. coli, yeast, insect cells or mammalian cells. Procedures for recombinant expression are described by Sambrook et al., Molecular Cloning: A Laboratory Manual (C.S.H.P. Press, NY 2d ed., 1989). Some forms of A.beta. peptide are also available commercially (e.g., American Peptides Company, Inc., Sunnyvale, Calif. and California Peptide Research, Inc. Napa, Calif.).

Therapeutic peptides also include longer polypeptides that include, for example, an active fragment of A.beta. peptide, together with other amino acids. Other amino acids can include those having adjuvant properties and those which serve to increase the stability of the peptide. For example, preferred peptides include fusion proteins comprising a segment of A.beta. fused to a heterologous amino acid sequence that induces a helper T-cell response against the heterologous amino acid sequence and thereby a B-cell response against the A.beta. segment. Such polypeptides can be screened for prophylactic or therapeutic efficacy in animal models. The A.beta. peptide, analog, active fragment or other polypeptide can be administered in associated or multimeric form or in dissociated form Therapeutic peptides also include multimers of monomeric and oligomeric immunogenic peptides. More generally, therapeutic peptides for use in the present invention produce or induce an immune response against a plaque, or more specifically, an amyloidogenic component thereof. Such peptides therefore include, but are not limited to, the component itself and variants thereof, analogs and mimetics of the component that induce and/or cross-react with antibodies to the component, as well as antibodies or T-cells that are specifically reactive with the fibril and/or amyloid peptide.

Induction of an immune response can be active, as when an immunogen is administered to induce antibodies or T-cells reactive with the component in a patient, or passive, as when an antibody is administered that itself binds to the fibril and/or amyloid peptide in the patient.

One general class of preferred anti-amyloid peptides consists of peptides that are derived from amyloid fibril proteins. As mentioned above, the hallmark of amyloid diseases is the deposition in an organ or organ of amyloid plaques consisting mainly of fibrils, which, in turn, are composed of characteristic fibril proteins or peptides. According to the present invention, such a fibril protein or peptide component is a useful peptide for inducing an anti-amyloid immune response. In accordance with this

aspect of the present invention, administration to an afflicted or susceptible individual of an immunostimulatory composition which includes the appropriate fibril protein or peptide, including homologs or fragments thereof, provides therapeutic or prophylaxis with respect to the amyloid disease.

Other formulations for treating hereditary forms of amyloidosis, discussed above, include compositions that produce immune responses against gelsolin fragments for treatment of hereditary systemic amyloidosis, mutant lysozyme protein (Alys), for treatment of a hereditary neuropathy, mutant alpha chain of fibrinogen (AfibA) for a non-neuropathic form of amyloidosis manifest as renal disease, mutant cystatin C (Acys) for treatment of a form of hereditary cerebral angiopathy reported in Iceland. In addition, certain hereditary forms of prion disease (e.g., Creutzfeldt-Jacob disease (CJD), Gerstmann-Strussler-Scheinker syndrome (GSS), and fatal familial insomnia (FFI)) are characterized by a mutant isoform of prion protein, PrP.sup.Sc. This protein can be used in therapeutic compositions for treatment and prevention of deposition of PrP plaques, in accordance with the present invention.

As discussed above, amyloid deposition, either systemic or focal, is also associated with aging. It is a further aspect of the present invention that such deposition can be prevented or treated by administering to susceptible individuals compositions consisting of one or more proteins associated with such aging. Thus, plaques composed of ATTR derived from wild type TTR are frequently found in heart tissue of the elderly. Similarly, certain elderly individuals may develop asymptomatic fibrillar focal deposits of A.beta. in their brains; A.beta. peptide treatment, as detailed herein may be warranted in such individuals. .beta..sub.2 microglobulin is a frequent component of corpora amylacea of the prostrate, and is therefore a further candidate peptide in accordance with the present invention.

By way of further example, but not limitation, there are a number of additional, non-hereditary forms amyloid disease that are candidates for treatment methods of the present invention. .beta..sub.2 microglobulin fibrillar plaques commonly develop in patients receiving long term hemodialysis or peritoneal dialysis. Such patients may be treated with therapeutic compositions directed to .beta..sub.2 microglobulin or, more preferably, immunogenic epitopes thereof, in accordance with the present invention.

Hormone-secreting tumors may also contain hormone-derived amyloid plaques, the

composition of which are generally characteristic of the particular endocrine organ affected. Thus such fibrils may be made up of polypeptide hormones such as calcitonin (medullary carcinoma of the thyroid), islet amyloid polypeptide (occurring in most patients with Type II diabetes), and atrial natriuretic peptide (isolated atrial amyloidosis). Compositions directed at amyloid deposits which form in the aortic intima in atherosclerosis are also contemplated by the present invention. For example, Westermark, et al. describe a 69 amino acid N-terminal fragment of Apolipoprotein A which forms such plaques (Westermark, et al. Am. J. Path. 147: 1186-92, 1995); therapeutic compositions of the present invention include immunological peptides directed to such a fragment, as well as the fragment itself.

The foregoing discussion has focused on amyloid fibril components that may be used as therapeutic peptides in treating or preventing various forms of amyloid disease.

The therapeutic peptide can also be an active carboxyl terminal fragment or analog of a naturally occurring or mutant fibril peptide or protein that contains an epitope that induces a similar protective or therapeutic immune response on administration to a human. Immunogenic fragments typically have a sequence of at least 3, 5, 6, 10 or 20 contiguous amino acids from a natural peptide. Analogs include allelic, species and induced variants. Analogs typically differ from naturally occurring peptides at one or a few positions, often by virtue of conservative substitutions. Analogs typically exhibit at least 80 or 90% sequence identity with natural peptides. Some analogs also include unnatural amino acids or modifications of N or C terminal amino acids. Examples of unnatural amino acids are alpha, alpha-disubstituted amino acids, N-alkyl amino acids, lactic acid, 4-hydroxyproline, (-carboxyglutamate, (-N,N,N-trimethyllysine, (-N-acetyllysine, O-phosphoserine, N-acetylserine, N-formylmethionine, 3-methylhistidine, 5-hydroxylysine, T-N-methylarginine. Such fragments, modified fragments or analogs may be used in therapeutic compositions of the present invention, if their immunoreactivity, specificity or efficacy is roughly equivalent to or greater than the corresponding parameters measured for the native amyloid fibril components.

Such peptides, proteins, or fragments, analogs and other amyloidogenic peptides can be synthesized by solid phase peptide synthesis or recombinant expression, according to standard methods well known in the art, or can be obtained from natural sources.

Therapeutic peptides may also be composed of longer polypeptides that include, for example, the active peptide fibril fragment or analog, together with other amino acids. Therapeutic peptides may also include multimers of monomeric and oligomeric immunogenic peptides or conjugates or carrier proteins, either non-covalently or covalently linked to the peptide.

In a preferred variation of the invention, an antigenic compound is thus provided using a Ligand Presenting Assembly (LPA as described in WO00/18791) backbone to link and present the carboxyl-terminal(s) of one or preferably at least two or several identical amyloid peptide(s) or fragments or derivatives thereof. Such peptides may be composed of naturally occurring amino acids or synthesized from unnatural amino acids. The peptides need not be aggregated to be operative or immunogenic as opposed to the prior art vaccines.

In a further variation, an immunogenic compound is provided using a Ligand Presenting Assembly backbone as described above to link and present the carboxyl-terminals of two or more non-identical amyloid peptide(s) or fragments or derivatives thereof. Such peptides may be composed of naturally occurring amino acids or synthesized from unnatural amino acids.

In a further variation, an immunogenic compound is provided using a Ligand Presenting Assembly backbone as described above to link and present the carboxyl-terminals of one or more identical or non-identical amyloid peptides or fragments or derivatives thereof and further provide a covalent link to yet another component.

In a preferred embodiment of the above, the Ligand Presenting Assembly backbone is according to prior art as described in WO00/18791. The Ligand Presenting Assembly is used to link two or more identical carboxyl-terminal fragments of amyloid beta (1-42) or derivatives thereof. The Ligand Presenting Assembly is further used to link the amyloid beta peptides to a T-cell epitope.

It is appreciated that immunological responses directed at other amyloid plaque components can also be effective in preventing, retarding or reducing plaque deposition in amyloid diseases. Such components may be minor components of fibrils or associated with fibrils or fibril formation in the plaques, with the caveat that components that are ubiquitous throughout the body, or relatively non-specific to the

amyloid deposit, are generally less suitable for use as therapeutic targets.

It is therefore a further discovery of the present invention that peptides that induce an immune response to specific plaque components are useful in treating or preventing progression of amyloid diseases. This section provides background on several exemplary amyloid plaque-associated molecules. Induction of an immune response against any of these molecules, alone or in combination with immunogenic therapeutic compositions against the fibril components described above or against any of the other non-fibril forming components described below, provides an additional anti-amyloid treatment regimen, in accordance with the present invention. Also forming part of the present invention are passive immunization regimens based on such plaque components, as described herein.

By way of example, synuclein is a protein that is structurally similar to apolipoproteins but is found in neuronal cytosol, particularly in the vicinity of presynaptic terminals. There are at least three forms of the protein, termed alpha, beta and gamma synuclein. Recently, it has been shown that alpha and .beta. synuclein are involved in nucleation of amyloid deposits in certain amyloid diseases, particularly Alzheimer's disease. (Clayton, D. F., et al., TINS 21(6): 249-255, 1998). More specifically, a fragment of the NAC domain of alpha and .beta. synuclein (residues 61-95) has been isolated from amyloid plaques in Alzheimer's patients; in fact this fragment comprises about 10% of the plaque that remains insoluble after solubilization with sodium dodecyl sulfate (SDS). (George, J. M., et al. Neurosci. News 1: 12-17, 1995). Further, both the full length alpha synuclein and the NAC fragment thereof have been reported to accelerate the aggregation of .beta.-amyloid peptide into insoluble amyloid in vitro. (Clayton, supra).

Immunization Procedures

The elicited antibodies present in the host having received the vaccine of the present invention bind at the carboxyl terminal region of A.beta.(1-42) or other sites such as A.beta.(10-21), A.beta.(13-21) or A.beta.(25-35) and have the ability to prevent amyloidogenesis. The vaccine of the present invention causes the generation of effective antiamyloidogenic antibodies in the vaccinated host.

A suggested immunization procedure is as follows:

a) prepare a vaccine by coupling a Ligand Presenting Assembly, amyloid peptides and a T-cell epitope. The amyloid peptide may be full length amyloid beta (1-42) , a carboxyl terminal fragment thereof, derivative or a related peptidomimetic. The coupling can be carried out as described in the prior art (WO00/18791)

b) immunize a host with the vaccine to generate an antibody in the host with a binding site capable of preventing fibrillogenesis, associated cellular toxicity and neurodegeneration. Preferably an antibody specifically directed towards the carboxyl-terminal region of amyloid beta (1-42).

Suitable pharmaceutically acceptable carriers include, without limitation, any non-immunogenic pharmaceutical adjuvants suitable for oral, parenteral, intravascular (IV), intraarterial (IA), intramuscular (IM), and subcutaneous (SC) administration routes, such as phosphate buffer saline (PBS).

The pharmaceutical carriers may contain a vehicle, which carries antigens to antigen-presenting cells. Examples of vehicles are liposomes, immune-stimulating complexes, microfluidized squalene-in-water emulsions, microspheres which may be composed of poly(lactic/glycolic) acid (PLGA). Particulates of defined dimensions (<5 micron) include, without limitation, oil-in-water microemulsion (MF59) and polymeric microparticules.

The carriers of the present invention may also include chemical and genetic adjuvants to augment immune responses or to increase the antigenicity of antigenic immunogens. These adjuvants exert their immunomodulatory properties through several mechanisms such as lymphoid cells recruitment, cytokine induction, and the facilitation of DNA entry into cells. Cytokine adjuvants include, without limitation, granulocyte-macrophage colony-stimulating factor, interleukin-12, GM-CSF, synthetic muramyl dipeptide analog or monophosphoryl lipid A. Other chemical adjuvants include, without limitation, lactic acid bacteria, Al(OH).sub.3, muramyl dipeptides and saponins.

The peptide may be coupled to a carrier that will modulate the half-life of the circulating peptide. This will allow the control on the period of protection. The peptide-carrier may also be emulsified in an adjuvant and administrated by usual immunization route.

The vaccine of the present invention will, for the most part, be administered parenterally, such as intravascularly (IV), intraarterially (IA), intramuscularly (IM), subcutaneously (SC), or the like. In some instances, administration may be oral, nasal, rectal, transdermal or aerosol, where the nature of the vaccine allows for transfer to the vascular system. Usually a single injection will be employed although more than one injection may be used, if desired. The vaccine may be administered by any convenient means, including syringe, trocar, catheter, or the like. Preferably, the administration will be intravascularly, where the site of introduction is not critical to this invention, preferably at a site where there is rapid blood flow, e.g., intravenously, peripheral or central vein. Other routes may find use where the administration is coupled with slow release techniques or a protective matrix.

The use of the vaccine of the present invention in preventing and/or treating Alzheimer's disease and other amyloid related diseases can be validated by raising antibodies against the LPA-peptide complex and testing them to see if they can effectively inhibit or prevent the fibrillogenesis of the natural amyloid peptide.

The compounds used to prepare vaccines in accordance with the present invention have the common structure of Formula I and II:

$R'\text{-}L\text{-}(P)_n$ (I)

$R'\text{-}L\text{-}(P')_n$ (II)
$(P^*)_n$

wherein

P is an amyloid peptide or fragment thereof or a peptide with substantial similarity to an amyloid peptide or a fragment thereof, e.g., A.beta.(e.g. 1-42), immunogenic fragments thereof, immunogenic derivatives thereof, protein conjugates thereof, immunogenic peptides thereof, and immunogenic peptidomimetics thereof.

n is a whole number higher than or similar to 1

L is a Ligand Presenting Assembly backbone e.g. as described in WO00/18791 e.g. $N(CH_2CO)_2$

R' is an N-terminal substituent, e.g.: hydrogen; lower alkyl groups, e.g., acyclic or cyclic having 1 to 8 carbon atoms, without or with functional groups, e.g., carboxylate, sulfonate and phosphonate; aromatic groups; heterocyclic groups; acyl groups, e.g., alkylcarbonyl, arylcarbonyl, sulfonyl and phosphonyl groups; and peptidic group. In one embodiment, R' is a human T cell epitope, e.g. the tetanus toxoid H-FNNFTVSFWLRVPKVSASHLE or a rodent T cell epitope H-QYIKANSKFIGITEL (as described by Valmori et al.).

P` is a an amyloid peptide as P described above and P* is an amyloid peptide as described above, but where P`is not identical to P*

In one embodiment, P is an amyloidogenic peptide capable of forming amyloid fibrils. In another embodiment, the preferred compounds are selected from the full-length peptides, A.beta.(1-43), A.beta.(1-42), and the lower homologues consisting of A.beta.(1-40), A.beta.(1-39), A.beta.(1-28).

In a preferred embodiment, the amyloid peptides are selected from a group of carboxyl terminal fragments of A.beta (1-42), e.g., A.beta.(38-42), A.beta.(37-42), A.beta.(36-42), A.beta.(35-42), A.beta.(34-42), A.beta.(33-42), A.beta.(32-42), A.beta.(31-42), A.beta.(30-42), A.beta.(29-42), A.beta.(28-42), A.beta.(27-42), A.beta.(26-42), A.beta.(25-42) or any modified versions or derivatives thereof. The peptides can be shortened further by removing one or more residues from either end or both ends

In another embodiment, the preferred compounds are selected from a group of short peptides, e.g., A.beta.(1-7), A.beta.(10-16), A.beta.(16-21), A.beta.(25-35), A.beta (17-37) or derivatives or modified versions thereof. The preferred compounds may also contain unnatural aminoacids or deletions or substitution of one or more residues in the naturally occurring sequence. In another embodiment, the preferred compounds are peptidomimetics of the above-said peptides.

In a further embodiment, the preferred compounds may be coupled with a carrier that will modulate the biodistribution, immunogenic property (e.g. a T-cell epitope) and the half-life of the compounds.

The present invention encompasses various types of immune responses triggered

using the vaccine of the present invention, e.g., amyloid therapies using the vaccine approach.

In accordance with a preferred embodiment of the present invention, some methods entail administering a dosage to a subject of a compound according to (I) or (II) that is effective to produce an immune response against an amyloid peptide characteristic of the amyloid disorder from which the subject suffers. In one preferred embodiment, the administration of the peptide will result in an antibody response that specifically binds to the 1-42 version of A.beta, preferably to residues 25-42, more preferably to residues 35-42.

In accordance with the present invention, there is also provided a vaccine which triggers a preferential TH-2 response or a TH-1 response, according to the type of immunization used. By inducing a TH-2 response, anti-inflammatory cytokine production such as IL-4, I1-10 and TGF-.beta., as well as the production of IgG 1 and IgG 2b antibody classes, are favoured. Such type of response would be preferred, as a major inflammatory response in the brain of the patients with AD would be avoided. On the other hand, with a preferred TH-1 response, a pro-inflammatory response with a production of inflammatory cytokines such as IL-1, Il-6, TNF and IFN gamma would be favoured. This type of response would more likely trigger activation of the macrophage population. These macrophages would then phagocytose any particulate deposits (such as plaques) via a complement-activated process as well as via antibody-mediated process. This approach would be beneficial to clear already organized senile plaques and prevent the formation of new fibrillary deposits.

Both approaches (i.e. TH-1 and TH-2) are of value. The antigen used could be the peptides which contain regions responsible for cellular adherence, i.e., region 10-16, regions responsible for the GAG binding site, i.e., 13-16, regions responsible for the .beta. sheet 16-21 or regions for 40-42. These peptides could be presented in such a way that either a preferential TH-1 or TH-2 response is obtained, depending on the type of adjuvant used, or depending on the route of administration of the vaccine. For example, a mucosal immunization via nasal administration is possible, since it is known that such a route of administration would favor a TH-2 response.

The present invention will be more readily understood by referring to the following example, which are given to illustrate the invention rather than to limit its scope.

EXAMPLE 1

An in vitro validation procedure can be used to test the effectiveness of vaccines derived from amyloid proteins according to the present invention. Immunisation in rabbits or mice can demonstrate that antibodies can be raised against A.beta. The antibodies produced can be tested, e.g. by ELISA techniques, to prove their specificity. Standard assays for fibrillogenesis, e.g. Thioflavine T, circular dichroism and solubility can also be used to demonstrate that they effectively can prevent the fibrillogenesis of natural A.beta.(1-42) in vitro.

This approach could also be used to establish which areas of the carboxyl terminal region of the A.beta (1-42) peptide are most effective to generate antibodies specifically directed towards the carboxyl terminus of A.beta (1-42) but not towards smaller versions, e.g. A.beta (1-40).

One way this could be performed is as follows:

a) rabbits or mice are immunized with a series of overlapping LPA-peptides generated from the A.beta.(1-42) terminal sequence, e.g., A.beta.(30-42), A.beta.(35-42), A.beta.(38-42) and also derivatives thereof where individual amino acids have been changed.

b) antisera are prepared from the immunized rabbits or mice.

c) these antisera are tested, e.g. in ELISA assays to see if the distribution of antibody response towards A.beta(1-42) that does not cross-react with A.beta (1-40)

CLAIMS

1. A method for preventing or treating an amyloid-related disease in a subject, comprising: administering to the subject an antigenic amount of a LPA-peptide (i.e. one or more peptides linked to a Ligand Presenting Assembly (LPA) backbone), wherein said LPA-peptide elicits the production of antibodies against said LPA-peptide and induces an immune response by said subject, thereby preventing or reducing amyloid-induced cellular toxicity or amyloid fibril formation

2. A method for preventing or treating an amyloid-related disease in a subject, comprising: administering to the subject an antigenic amount of a peptide, wherein said peptide elicits the production of antibodies against the carboxyl terminus of said peptide and induces an immune response by said subject, thereby preventing or reducing amyloid-induced cellular toxicity or amyloid fibril formation.

3. The method of claim 1, wherein said LPA-peptide comprises at least one region of an amyloid protein.

4. The method of claim 3, wherein said region is being selected from the group consisting of: C-terminal region, beta sheet region, cytotoxic region, GAG-binding site region, macrophage adherence region, immunogenic fragments thereof, protein conjugates thereof, immunogenic derivative peptides thereof, immunogenic peptides thereof, and immunogenic peptidomimetics thereof.

5. The method of claim 3, wherein said LPA-peptide comprises at least two different regions of the same amyloid protein.

6. The method of claim 3, wherein said LPA-peptide comprises regions of at least two different amyloid protein.

7. The method of claim 3, wherein said LPA-peptide further comprises an acid functional group, or a pharmaceutically acceptable salt or ester form thereof.

8. The method of claim 3, wherein said LPA- peptide further comprises a base functional group, or a pharmaceutically acceptable salt form thereof.

9. The method of claim 3, wherein said amyloid protein is selected from the group consisting of: Serum Amyloid A protein (ApoSSA), immunoglobulin light chain, immunoglobulin heavy chain, ApoA1, transthyretin, lysozyme, fibrinogen .alpha. chain, gelsolin, cystatin C, Amyloid beta protein precursor (beta.-APP), Beta.sub.2 microglobulin, prion precursor protein (PrP), atrial natriuretic factor, keratin, islet amyloid polypeptide, or synuclein or any modified peptides, mutant proteins, protein fragments or proteolytic peptides thereof.

10. The method of claim 3, wherein said amyloid protein is amyloid beta (1-43), amyloid beta (1-42), amyloid beta (1-41), amyloid beta (1-40), amyloid beta (1-39), amyloid beta (1-38) or a fragment thereof.

11. The method of claim 1, wherein at least one peptide is a carboxyl terminal fragment of amyloid beta (1-43), amyloid beta (1-42), amyloid beta (1-41), amyloid beta (1-40), amyloid beta (1-39), or amyloid beta (1-38)

12. The method of claim 10, wherein said amyloid protein is modified by removing or substituting at least one amino acid residue with another amino acid or non-amino acid fragment.

13. The method of claim 11, wherein said carboxyl terminal fragment is modified by removing or substituting at least one amino acid residue with another amino acid or non-amino acid fragment

14. The method of claim 9, wherein said amyloid protein is modified by removing or substituting at least one amino acid residue with another amino acid or non-amino acid fragment

15. A method for preventing or treating an amyloid-related disease in a subject, comprising: administering to the subject an antigenic amount of a compound having Formula I: $R'\text{-}L\text{-}(P)_n$ (I)
wherein P is a full length or fragment amyloid peptide, e.g., Serum Amyloid A protein (ApoSSA), immunoglobulin light chain, immunoglobulin heavy chain, ApoA1, transthyretin, lysozyme, fibrinogen .alpha. chain, gelsolin, cystatin C, Amyloid beta protein precursor (beta.-APP), Beta.sub.2 microglobulin, prion precursor protein (PrP), atrial natriuretic factor, keratin, islet amyloid polypeptide, or synuclein or an

immunogenic fragments thereof, immunogenic derivatives thereof, protein conjugates thereof, immunogenic peptides thereof, immunogenic peptidomimetics thereof or a peptide with substantial similarity to an amyloid peptide or a fragment thereof; n is a whole number higher than or similar to 1; L is a backbone as described in WO00/18791 e.g. $N(CH_2CO)_2$; R' is an N-terminal substituent, e.g.: hydrogen; lower alkyl groups, e.g., acyclic or cyclic having 1 to 8 carbon atoms, without or with functional groups, e.g., carboxylate, sulfonate and phosphonate; aromatic groups; heterocyclic groups; acyl groups, e.g., alkylcarbonyl, arylcarbonyl, sulfonyl and phosphonyl groups; and peptidic group.

16. The method of claim 15, wherein R' is a T cell epitope.

17. The method of claim 16, wherein R' is a human T cell epitope, e.g. the tetanus toxoid H-FNNFTVSFWLRVPKVSASHLE or a rodent T cell epitope, e.g. H-QYIKANSKFIGITEL

18. The method of claim 16, wherein P is amyloid beta (1-43), amyloid beta (1-42), amyloid beta (1-41), amyloid beta (1-40), amyloid beta (1-39), amyloid beta (1-38) or a fragment thereof.

19. The method of claim 16, wherein P is a carboxyl terminal fragment of amyloid beta (1-43), amyloid beta (1-42), amyloid beta (1-41), amyloid beta (1-40), amyloid beta (1-39), or amyloid beta (1-38)

20. The method of claim 18, wherein said amyloid protein is modified by removing or substituting at least one amino acid residue with another amino acid or non-amino acid fragment.

21. The method of claim 19, wherein said carboxyl terminal fragment is modified by removing or substituting at least one amino acid residue with another amino acid or non-amino acid fragment

22. A method for preventing or treating an amyloid related disease in a subject, comprising administering to the subject an antigenic amount of a compound of Formula II:

$R'\text{-}L\text{-}(P')_n$ (II)

$(P^*)_n$

wherein P` and P* are full length or fragment amyloid peptides, e.g., Serum Amyloid A protein (ApoSSA), immunoglobulin light chain, immunoglobulin heavy chain, ApoA1, transthyretin, lysozyme, fibrinogen .alpha. chain, gelsolin, cystatin C, Amyloid beta protein precursor (beta.-APP), Beta.sub.2 microglobulin, prion precursor protein (PrP), atrial natriuretic factor, keratin, islet amyloid polypeptide, or synuclein or an immunogenic fragments thereof, immunogenic derivatives thereof, protein conjugates thereof, immunogenic peptides thereof, immunogenic peptidomimetics thereof or a peptide with substantial similarity to an amyloid peptide or a fragment thereof, but where P`is not identical to P*; n is a whole number higher than or similar to 1; L is a backbone as described in WO00/18791 e.g. $N(CH_2CO)_2$; R' is an N-terminal substituent, e.g.: hydrogen; lower alkyl groups, e.g., acyclic or cyclic having 1 to 8 carbon atoms, without or with functional groups, e.g., carboxylate, sulfonate and phosphonate; aromatic groups; heterocyclic groups; acyl groups, e.g., alkylcarbonyl, arylcarbonyl, sulfonyl and phosphonyl groups; and peptidic group.

23. The method of claim 22, wherein R' is a T cell epitope.

24. The method of claim 23, wherein R' is a human T cell epitope, e.g. the tetanus toxoid H-FNNFTVSFWLRVPKVSASHLE or a rodent T cell epitope, e.g. H-QYIKANSKFIGITEL

25. The method of claim 23, wherein P is amyloid beta (1-43), amyloid beta (1-42), amyloid beta (1-41), amyloid beta (1-40), amyloid beta (1-39), amyloid beta (1-38) or a fragment thereof.

26. The method of claim 23, wherein P is a carboxyl terminal fragment of amyloid beta (1-43), amyloid beta (1-42), amyloid beta (1-41), amyloid beta (1-40), amyloid beta (1-39), or amyloid beta (1-38)

27. The method of claim 25, wherein said amyloid protein is modified by removing or substituting at least one amino acid residue with another amino acid or non-amino acid fragment.

28. The method of claim 26, wherein said carboxyl terminal fragment is modified by removing or substituting at least one amino acid residue with another amino acid or non-amino acid fragment

29. The method of claim 15, wherein said compound elicits the production of antibodies against said compound, and induces an immune response by said subject, thereby preventing or reducing amyloid-induced cellular toxicity or amyloid fibril formation.

30. The method of claim 22, wherein said compound elicits the production of antibodies against said compound, and induces an immune response by said subject, thereby preventing or reducing amyloid-induced cellular toxicity or amyloid fibril formation.

31. The method of claim 15, wherein said all-D peptide further comprises an acid functional group, or a pharmaceutically acceptable salt or ester form thereof.

32. The method of claim 15, wherein said all-D peptide further comprises a base functional group, or pharmaceutically acceptable salt form thereof.

33. The method of claim 22, wherein said all-D peptide further comprises an acid functional group, or a pharmaceutically acceptable salt or ester form thereof.

34. The method of claim 22, wherein said all-D peptide further comprises a base functional group, or pharmaceutically acceptable salt form thereof.

35. A composition for preventing or treating an amyloid-related disease in a subject, comprising: an antigenic amount of a LPA-peptide (i.e. one or more peptides linked to a Ligand Presenting Assembly (LPA) backbone), wherein said LPA-peptide elicits the production of antibodies against said LPA-peptide and induces an immune response by said subject, thereby preventing or reducing amyloid-induced cellular toxicity or amyloid fibril formation

36. The composition of claim 35, wherein said LPA-peptide comprises an amyloidogenic protein or protein fragment such as Serum Amyloid A protein (ApoSSA), immunoglobulin light chain, immunoglobulin heavy chain, ApoA1, transthyretin, lysozyme, fibrinogen .alpha. chain, gelsolin, cystatin C, Amyloid beta protein precursor (beta.-APP), Beta.sub.2 microglobulin, prion precursor protein (PrP), atrial natriuretic

factor, keratin, islet amyloid polypeptide, or synuclein or any modified peptides, mutant proteins, protein fragments or proteolytic peptides thereof.

37. The composition of claim 35, wherein said amyloid protein is amyloid beta (1-43), amyloid beta (1-42), amyloid beta (1-41), amyloid beta (1-40), amyloid beta (1-39), amyloid beta (1-38) or a fragment thereof.

38. The composition of claim 35, wherein at least one peptide is a carboxyl terminal fragment of amyloid beta (1-43), amyloid beta (1-42), amyloid beta (1-41), amyloid beta (1-40), amyloid beta (1-39), or amyloid beta (1-38)

39. The composition of claim 37, wherein said amyloid protein is further modified by removing or substituting at least one amino acid residue with another amino acid or non-amino acid fragment.

40. The composition of claim 38, wherein said amyloid protein is further modified by removing or substituting at least one amino acid residue with another amino acid or non-amino acid fragment.

41. The composition of claim 36, wherein said LPA-peptide further comprises an acid functional group, or a pharmaceutically acceptable salt or ester form thereof.

42. The composition of claim 36, wherein said LPA-peptide further comprises a base functional group, or pharmaceutically acceptable salt form thereof.